Supporting Information

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SI Materials and Methods

Strain List. NC1400 *pRN2003[pdat-1::GFP rol-6(su1006)]; juIs96 [punc-25::GFP lin-15(+)]; eri-1(mg366); lin-15B(n744)*
NC1404 *juIs14[pacr-2::GFP lin-15(+)]; eri-1(mg366); lin-15B(n744)*
NC1405 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366); lin-15B(n744)*
NC1407 *juIs96[punc-25::GFP lin-15(+)]; ced-4(n1162); eri-1(mg366); lin-15B(n744)*
NC1420 *myo-3(st386); eri-1(mg366); lin-15B(n744); stEx30 [myo-3::GFP rol-6(su1006)]*
NC1436 *adIs1240[eat-4::GFP lin-15(+)]; eri-1(mg366); lin-15B(n744)*
NC1437 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366); crt-1(bz30); lin-15B(n744)*
NC1439 *mgIs42[tph-1::GFP rol-6(su1006)]; eri-1(mg366); lin-15B(n744)*
NC1758 *juIs96[punc-25::GFP lin-15(+)]; ced-9(n1950); eri-1(mg366)*
NC1778 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366)* drp-1(tm1108)
NC1635 *coq-1(ok749)/dpy-5(e61); juIs14[acr-2::GFP lin-15(+)]*
NC1660 *coq-1(ok749)/dpy-5(e61); juIs96[punc-25::GFP lin-15(+)]*
NC1647 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366) ced-3 (n717)*
NC1941 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366); egl-1 (n1084n3082)*
NC1959 *juIs96[punc-25::GFP lin-15(+)]; coq-2(ok1066)/hT2*
NC1974 *juIs96[punc-25::GFP lin-15(+)]; coq-3(ok506)/nT1*
NC1993 *coq-2(ok1066)/hT2; juIs14[pacr-2::GFP lin-15(+)]*
NC1999 *juIs96[punc-25::GFP lin-15(+)]; ced-4(n1162); coq-3 (ok506)/nT1*
NC2020 *coq-2(ok1066)/hT2; juIs14[pacr-2::GFP lin-15(+)]; wdEx658[punc-25::mcherry unc-119(+)]*
NC2026 *juIs96[punc-25::GFP lin-15(+)]; eri-1(mg366); egl-1 (ok1418)*

Strain Maintenance. N2 (wild-type), *ced-3(n717), ced-4(n1162), ced-9(n1950gf), coq-1(ok749), coq-2(ok1066), coq-3(ok506), crt-1 (bz30), egl-1(ok1418), and egl-1(n1084n3082),* and the GFP reporter strains *acr-2::GFP(juIs14)* (1), *eat-4::GFP(adIs1240)* (2), *myo-3::MYO-3-GFP(stEx30)* (3), *unc-25::GFP(juIs76)* (4), and *tph-1::GFP (mgIs42)* (5) were obtained from the Caenorhabditis Genetics Center (University of Minnesota, Minneapolis, MN). *drp-1(tm1108)* was provided by D. Xue (University of Colorado, Boulder, CO) (6). *pdat-1::GFP(pRN2003)* was obtained from R. Blakely (Vanderbilt University, Nashville, TN) (7). *coq-1(ok749)* was outcrossed four times, maintained in trans to *dpy-5(e61)*, and genotyped by PCR: 5′-catcgaatttaggacgaatgac (forward), 5′-cttcacgcagaggtcagagtg (reverse), 5′-ccaacttgctgacgatcttcttg (poison). PCR conditions were as follows: denaturing, 94 °C for 5 min; cycling, 94 °C for 30 s, 50 °C for 45 s, 72 °C for 3 min, 35 times; final extension, 72 °C for 7 min. *coq-1* wild-type bands are 2.5 kb and ≈900 bp and *coq-1* mutant band is ≈600 bp. *coq-1(ok749); unc-25::GFP(juIs76)* and *coq-1(ok749); acr-2::GFP(juIs14)* animals were used to examine GABA and cholinergic neurons in *coq-1* mutant animals, respectively.

GABA neuron degeneration was observed in *coq-1(ok749)* maintained at 15 °C. *egl-1(ok1418), egl-1(n1084n3082)*, and *ced-9 (n1950)* were verified by sequencing in *eri-1(mg366); unc-25::GFP(juIs76)* genetic backgrounds. Cholinergic neurons marked with *acr-2::GFP* (1) were quantified in the same manner, and no significant degeneration was detected. Additional neuron classes were examined (S4), but no differences between control (empty vector) and *coq-1* RNAi knockdown animals were observed in these neurons. For GFP knockdown in reporter strains (S4), quantification of fluorescence in Z series of confocal optically sectioned images was done by using histogram analysis in ImageJ (8).

pMLH41 was constructed by ligating a 1.8-kb PCR amplicon of the *unc-25* promoter from pSC392 (a generous gift from Yishi Jin, University of California, San Diego, CA) into *punc-4::mcherry* (9) by using 5′-SphI and 3′-AscI. The *unc-119* minigene from pDP#MM051 (10) was inserted into the vector backbone between PacI and ApaI.

PCR Assay To Detect *eri-1(mg366).* Single-worm PCR was performed with primers 5′-gataaaacttcggaacatatggggc (forward) and 5′-actgatgggtaaggaatcgaagacg (reverse); the *eri-1* PCR amplicon (223 bp) is distinguishable on a 2% agarose gel from the wild-type band (200 bp). *eri-1 ced-3* recombinants were created by crossing *eri-1 unc-22* heterozygous males into *ced-3* hermaphrodites. Non-Unc animals were PCR-genotyped for *eri-1/eri-1*, and animals lacking *unc-22* progeny were verified by sequencing as *eri-1 ced-3* (11).

Treatments with Coenzyme Q (CoQ). CoQ_{10} (Sigma) was dissolved in 0.06% Tween-80 (12) and added to media before pouring plates. Tween-80 (0.06%) alone had no effect on degeneration and was used as the normalization control for those experiments.

Combinatorial RNAi

Cultures for combinatorial-RNAi experiments (i.e., *coq-1 + drp-1*, *coq-1 + kap-1, coq-1 + fzo-1*) were grown separately and then mixed before plating. *kap-1* is an unrelated gene that has no visible phenotype by RNAi and was used as a control for potential competitive effects of combining two RNAi constructs (Fig. S8).

ATP Measurement

Crude mitochondria were isolated from lysates (13) obtained from a mixed population of *eri-1; unc-25::GFP(juIs76)* animals treated with either empty vector (EV) or *coq-1* RNAi. A Roche Cell-Titer Glow Luminescent Cell Viability Assay kit was used to measure ATP levels with a FluorStar Optima luminometer. ATP levels were normalized to protein concentrations and represented in arbitrary units. A Bio-Rad protein assay kit was used to measure protein concentrations in a BioRad3 spectrophotometer. Each measurement was replicated in three independent experiments.

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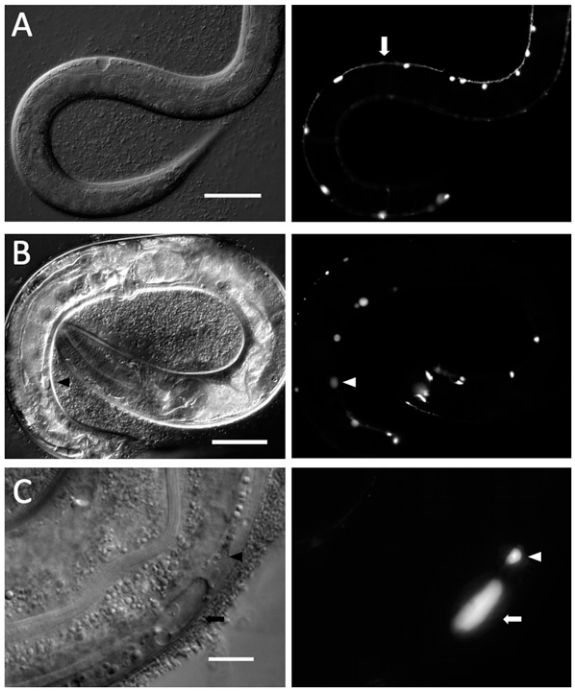


Fig. S1. GABA neuron degeneration in *coq-1(ok749)* animals. DIC (*Left*) and GFP (*Right*) views of *unc-25::GFP*-labeled GABA neurons in *coq-1(ok749)* at the L3 (*A*) and adult (*B*) stages. Arrow denotes intact ventral nerve cord (VNC) in L3 larva (*A*) and arrowhead marks swollen GABAergic motor neuron cell body in adult (*B*). (*C*) Enlarged (arrow) and normal (arrowhead) GABAergic cell bodies in the VNC. (Scale bars: *A* and *B*, 20 μm; *C*, 10 μm.)

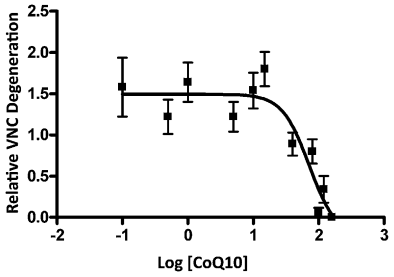
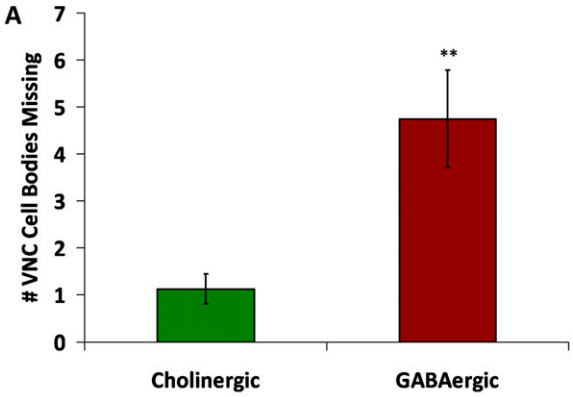


Fig. S2. *coq-1* RNAi-induced GABA neuron degeneration is rescued by treatment with exogenous CoQ_{10}. Dose–response curve showing rescue of GABA neuron degeneration with exogenous CoQ_{10} (n = 30, three independent experiments). EC_{50} = 72 mg/mL. After 5 d of RNAi treatment, animals were scored for percent VNC degeneration as in Fig. 2. Relative VNC degeneration was calculated by normalizing percent degeneration at each concentration of CoQ_{10} to percent degeneration in Tween-80 alone control plates for each separate experiment.

A



B

animal #	# cholinergic neurons missing (*acr-2* ::GFP)	% cholinergic neurons missing (*acr-2* ::GFP)	# GABA neurons missing (*unc-25* ::mcherry)	% GABA neurons missing (*unc-25* ::mcherry)
1	2	7%	0	0%
2	0	0%	7	54%
3	1	3%	1	8%
4	1	3%	3	23%
5	2	7%	1	8%
6	1	3%	2	15%
7	0	0%	6	46%
8	0	0%	1	8%
9	5	17%	7	54%
10	0	0%	13	100%
11	0	0%	3	23%
12	1	3%	9	69%
13	1	3%	0	0%
14	2	7%	12	92%
15	1	3%	4	31%
16	1	3%	7	54%

Fig. S3. GABA neurons are more sensitive than neighboring cholinergic neurons to *coq-2* depletion. (*A*) The number of absent cholinergic (*acr-2::GFP*) and GABAergic (*unc-25::mcherry*) neurons (VA2-VA11 and VD3-VD11, respectively) in adult *coq-2* animals.**$P < 0.002$; $n = 16$. (*B*) Original data collected for Fig. 2*K* and Fig. S3*A*.

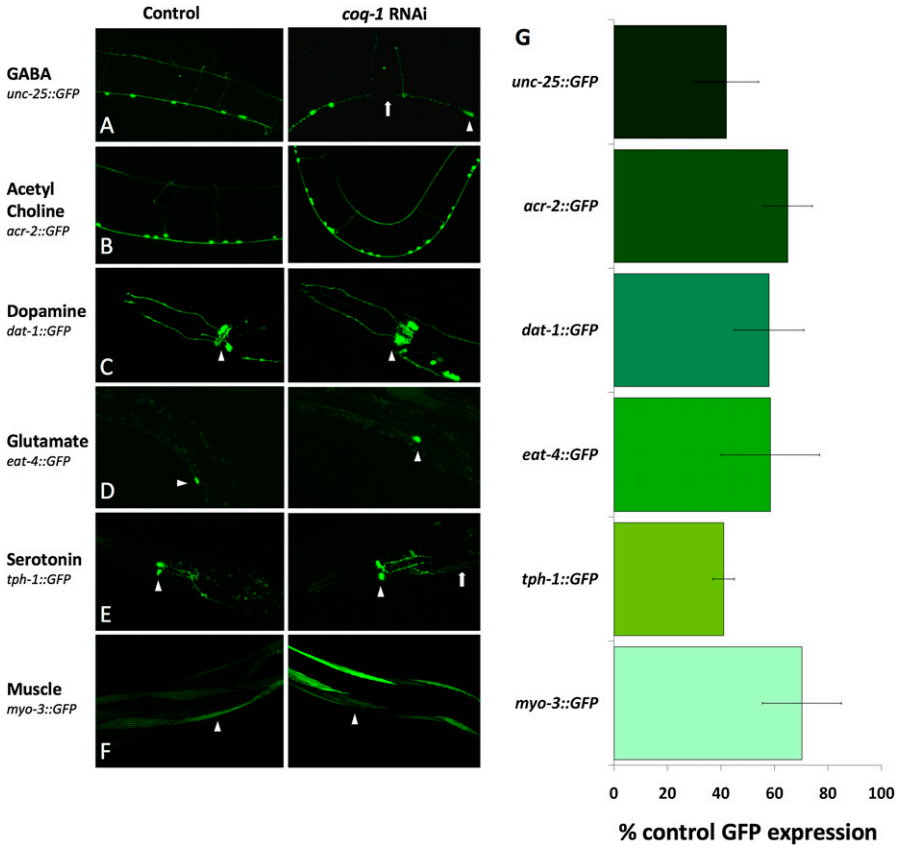


Fig. S4. *coq-1* RNAi triggers preferential degeneration of GABA neurons. Confocal images (40×) of empty vector treated control or *coq-1* RNAi-treated animals expressing GFP reporters specific for different neurotransmitter classes of neurons. (*A*) GABAergic neurons detected by *unc-25::GFP*. *coq-1* RNAi-treated animals show both VNC degeneration (arrow) and swollen neurons (arrowhead). Other classes of neurons and muscle cells do not show degeneration in age-matched animals subject to *coq-1* RNAi-treatment for the same period. (*B*) Cholinergic neurons, *acr-2::GFP*. (*C*) Dopaminergic neurons, *dat-1::GFP*. Arrowhead, CEP neuron. (*D*) Glutamatergic neurons, *eat-4::GFP*. Arrowheads point to AVM (*Left*) and ALM (*Right*). (*E*) Serotonergic neurons, *tph-1::GFP*. NSM cell body (arrowheads) and HSN axonal projection (arrow) are shown. (*F*) Muscle, *myo-3::GFP*. Arrowheads point to body wall muscle cells. (*G*) Sensitivity to RNAi knockdown in each GFP-labeled cell type was determined by measuring GFP fluorescence after RNAi against GFP. GFP fluorescence intensity values were obtained from a through focus Z-series collected in a confocal microscope and quantified by histogram analysis in ImageJ (n = 6, avg ± SD). Fluorescence intensities for each GFP strain were normalized to measurements obtained from a control (RNAi with empty vector) to calculate % control GFP expression.

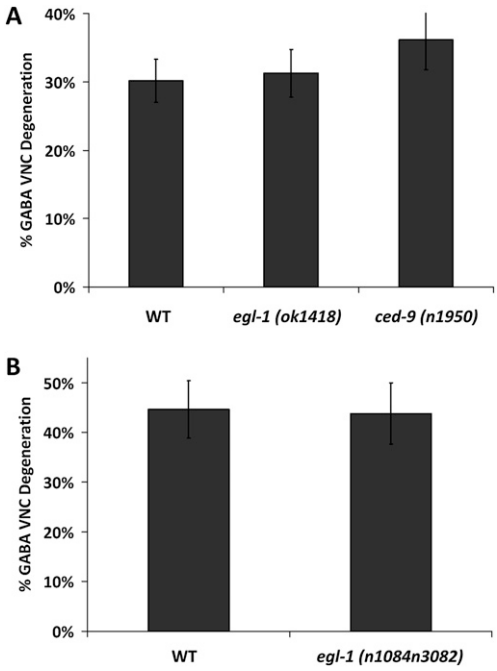


Fig. S5. Apoptotic genes *egl-1* and *ced-9* are not required for *coq-1* RNAi-induced GABA neuron degeneration. (*A*) *egl-1(ok1418)* and *ced-9(n1950)* were assayed for GABA neuron degeneration after *coq-1* knockdown as in Fig. 2 ($n > 40$). Results with *egl-1* and *ced-9* do no differ significantly from the wild-type control in these experiments. (*B*) In a separate experiment, another *egl-1* loss-of-function allele, *n1084n3082*, was treated with *coq-1* RNAi and assayed for GABA neuron degeneration ($n > 20$), with no significant difference from the wild-type control.

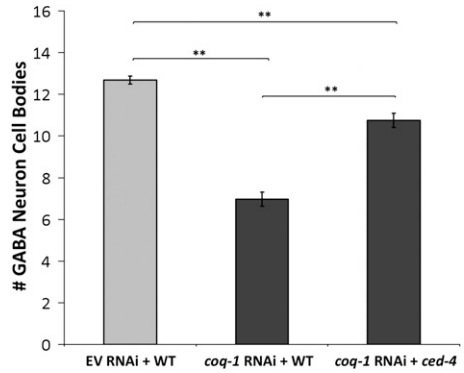


Fig. S6. *ced-4* suppresses degeneration of GABA neuron cell bodies in *coq-1* RNAi-treated animals. *unc-25::GFP*-positive GABA neurons in the VNC interval from VD3-DD11 (13 total cells) were counted for *coq-1* RNAi-treated wild-type (WT) and *ced-4(n1162)* and for the negative control of wild-type treated with empty vector (EV RNAi + WT), $^{**}P < 0.0001$, $n > 50$. All strains include the *eri-1(mg366)* mutant allele.

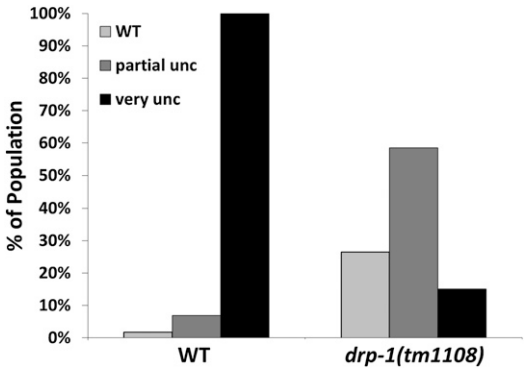


Fig. S7. Uncoordinated (Unc) movement induced by *coq-1* RNAi is suppressed by *drp-1*. Movement assay of wild-type (WT) and *drp-1(tm1108)* mutant animals treated with *coq-1* RNAi. Results shown here were obtained from *coq-1* RNAi-treated animals containing *unc-25::GFP; eri-1(mg366)*. Adults were tapped once on the head and the tail and scored for movement: "partial unc" represents worms that exhibit uncoordinated movement, and "very unc" indicates worms that are unable to move. $n > 100$.

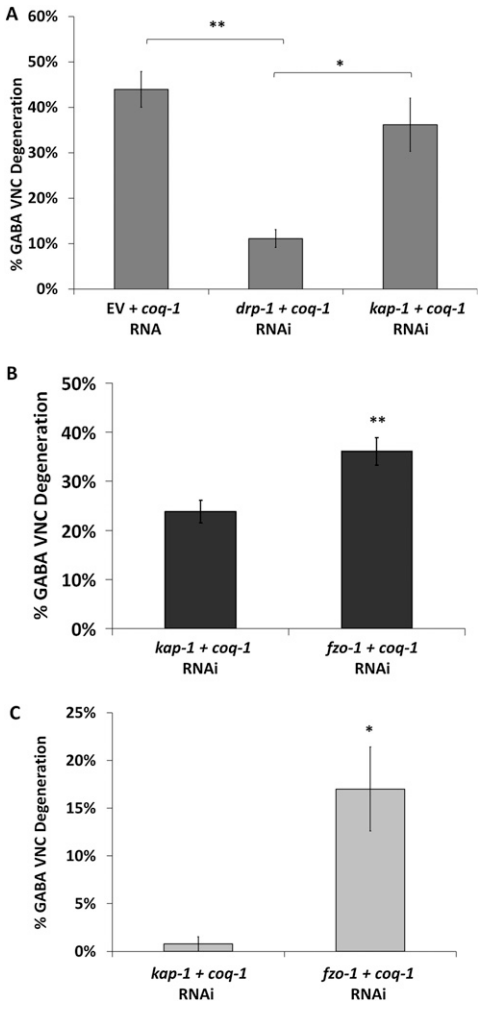


Fig. S8. RNAi of mitochondrial fission and fusion genes influences GABA neuron degeneration induced by *coq-1* RNAi. (*A*) Double RNAi with empty vector (EV) and *coq-1* results in GABA neuron degeneration (≈44%). Double RNAi of *coq-1* with the mitochondrial fission gene *drp-1* results in significantly less GABA neuron degeneration than EV + *coq-1* RNAi control. Double RNAi with a randomly selected control gene *kap-1* (kinesin-associated protein) did not inhibit *coq-1* RNAi-induced GABA neuron degeneration. $n = 60$, $^{**}P < 0.0001$; $^{*}P < 0.0003$. (*B*) Combinatorial RNAi with *fzo-1* (mitochondrial outer membrane fusion gene) and *coq-1* enhance the GABA neurodegeneration observed with *coq-1* and an unrelated gene, *kap-1*, $n > 100$, $^{**}P < 0.0001$. (C) *fzo-1* RNAi enhances *coq-1* knockdown-induced GABA neuron degeneration in animals lacking the RNAi sensitive mutation *eri-1(mg366)*. $n > 15$, $^{*}P < 0.002$ (Student's *t* test).

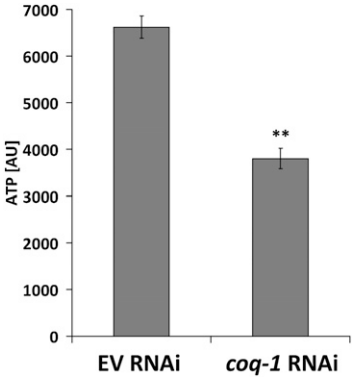


Fig. S9. Mitochondrial ATP levels are reduced in CoQ-depleted animals. ATP levels in mitochondria isolated from whole animal lysates are reduced in *coq-1* RNAi-treated animals versus empty vector-treated control (EV RNAi). $^{**}P < 0.0008$, $n = 3$. Each sample contained 0.2 μg of total protein. Relative ATP levels are denoted (AU, arbitrary units). ATP levels were measured by using a luminescence assay and normalized to protein concentration (*SI Materials and Methods*).

Movie S1. *coq-1* RNAi induces progressive loss of motor function during larval development. Animals treated with *coq-1* RNAi in early larval stages display wild-type locomotion. As animals progress into late larval (L4) and adult stages, however, body movement is increasingly impaired or uncoordinated (Unc) with older adults appearing largely immobilized.

Movie S1